Human adipose stem cells: properties and use in clinical applications

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LAURA MÄKINEN: IHMISEN RASVAKUDOKSEN KANTASOLUJEN KÄYTTÖ KLIINISISSÄ SOVELLUKSISSA

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Aikuisen rasvakudoksen kantasolut (adipose stem cells, ASCs) ovat multipotentteja, mesenkymaalisia kantasoluja. Niillä on useita kliinistä käyttöä suosivia ominaisuuksia, kuten kyky erilaistua luu-, rusto- ja rasvakudoksen suuntaan, hyvä jakautumiskyky in vitro -olosuhteissa sekä moniin muihin kantasolulähteisiin verrattuna helppo ja vaivaton eristysmenetelmä. Nykyisiä soluviljelymenetelmiä tulisi kuitenkin kehittää, mikäli pyritään kantasoluterapian käyttöön rutiinihoitona.

Työssä tutkittiin rasvakudoksen kantasolujen ominaisuuksia eri viljelyolosuhteissa, jotta voitaisiin kehittää kantasoluhoitojen turvallisuutta ja laatua. Tutkimus toteutettiin eristämällä neljän luovuttajan rasvakudosnäytteistä kantasolut, ja toistamalla samat toimenpiteet ja kokeet eri olosuhteissa: 1) ihmisen seerumia sisältävä kasvatusliuos, 2) naudan seerumia sisältävä kasvatusliuos sekä 3) täysin seerumiton ja eläinperäisistä aineista vapaa kasvatusliuos. Työn tarkoituksena oli tutkia kasvatusliuoksen makromolekulaarisen täytön (macromolecular crowding, MMC) eli Ficollin lisäyksen vaikutusta soluihin ja kykyä tukea soluja tuottamaan luonnollista mikrotason elinympäristöä laboratorio-olosuhteissa. Työssä tutkittiin edellä mainittujen olosuhteiden vaikutusta solujen morfologiaan, lisääntymiseen, immunofenotyyppiin, DNA:n määrään ja metaboliseen aktiivisuuteen sekä erilaistuskykyyn.

Tutkimuksessa havaittiin, että solujen pintaproteiinien ilmentyminen ei muuttunut merkittävästi, mutta vaikutukset erilaistumiseen, morfologiaan ja erityisesti jakautumiseen olivat huomattavia. Työn perusteella MMC-menetelmä seerumittomissa olosuhteissa ei sovellu rasvakudoksen kantasoluille, sillä pitkäkestoinen viljely ei ole mahdollista. MMC-menetelmää voidaan kuitenkin käyttää tukemaan erilaistumista seerumia sisältävissä kasvatusolosuhteissa.

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1 Introduction

Autologous, multipotent stem cells have enormous potential when it comes to clinical research and tissue engineering. One source of such cells is human adipose tissue. Adipose stem/stromal cells (ASCs) are multipotent cells of mesenchymal origin, and they have the ability to differentiate toward multiple mesenchymal cell types, such as adipose, bone and cartilage cells. After their discovery in the beginning of the century, ASCs have quickly proven to have several beneficial characteristics that favor their usage in cell therapy. They are abundant and accessible just beneath the skin in subcutaneous tissue and possess wide differentiation capacity as well as promising cultivation properties, such as relatively high in vitro-proliferation rate (1,2). The field of tissue engineering has grown tremendously over the past ten to twenty years and researchers have worked hard to master the properties of stem cells and to enable their utilization in every day medicine. However, much remains to be discovered and at the moment, despite their promising features and the increasing number of clinical studies using ASCs, we only have a grasp of the basics of their differentiation. Especially the molecular and transcriptional events of adipose stem cells remain poorly understood.

When it comes to tissue engineering with medical purposes, it is clear that the current methods for cultivation are not adequate to provide reliable results or to ensure the safety of the final product. Traditionally, in vitro culture medium has been supplemented with fetal bovine serum (FBS) due to its availability, low price and capability to support cell growth in culture. In clinical therapy, however, using reagents of animal origin should be avoided to minimize complications in result of interspecific interplay. Thus FBS and other similar products are not ideal for clinical use (3,4). To avoid these issues while attempting to satisfy the needs of the cultured cells, alternative methods have been developed: cells have been cultured with human serum (HS) or platelet-derived supplements (5) as well as with xeno- and serum-free (XF/SF) supplements, developed only quite recently (6). Replacing all animal-derived components with these XF/SF reagents is still relatively marginal and thus there are no appropriate, standardized methods available for cultivation of stem cells with the exception of only a few cell types.

Unfortunately, the question around serum is not the only problem concerning culture conditions. Choosing the components of a culture medium is based on two different needs: the cells requiring an environment close to their natural in vivo-habitat, and, on the other hand, the scientist trying to

maintain beneficial in vitro-conditions. In practice this means using only vital components to fulfill the needs of the cells, which extremely poorly, however, matches the concentrations in living tissues (7). This is the reality especially in regard to the overall concentration of the medium. The protein components mimicking plasma are alone responsible for the macro-environment of the culture dish and so the macro-molecular concentration may only be fractions of the corresponding in reality. The situation is quite off-balanced since it is well known that the macro-environment affects the formation and remodeling of extracellular matrix (ECM) and so has an influence on many key elements, including formation of cytoskeleton, enzymatic reactions, adhesion and migration (7,8).

As to how much the macro-environment affects the growth and development of cultured cells is not fully understood but especially the joint effect of different factors – different medium components on different cell types – is poorly known. A method altering the concentration of the medium called macromolecular crowding (MMC) has only been studied with bone marrow mesenchymal stem cells in more traditional culture media containing FBS (7), whereas the possibilities in regard to cell therapy appear to be promising with adipose stem cells. ASCs on the other hand have well-defined XF/SF culturing protocols. Managing to combine these two might just get us closer to reality – bringing in vivo in vitro that is.

2 Human adipose stem cells: discovery, definition and promising features

Human adult adipose stem cells were discovered as recently as in 2001. It was plastic surgeon Dr. Marc Hendrick and his coworkers who tried to culture tissue material collected in a liposuction but, by accident, ended up killing most of the cells. Only a small population survived and appeared to be unusually resistant. The finding quickly proved to be remarkable since it was soon understood that these cells could be cultured, differentiated and modified with relative ease, and more material could be acquired effortlessly. Since Zuk *et al.* (9) posted their findings, the interest in and number of clinical studies using ASCs has been gradually and steadily rising.

Stem cell is defined as an ”undifferentiated cell that can continue dividing indefinitely, throwing off daughter cells that can either commit to differentiation or remain a stem cell in the process of self-renewal” (10). In humans, such cells can be divided into two categories: embryonic stem cells derived from the inner cell mass of an early embryo and capable of giving rise to all the cell types in

an individual, and adult stem cells found in various tissues in a fully developed human being but having more limited differentiation possibilities. Adult stem cells can further be divided according to the germ layer they originate from: ectoderm, endoderm and mesoderm, the latter giving rise to mesenchyme, a primitive meshwork of embryonic connective tissue. Like all connective tissues, adipose tissue is derived from mesenchyme thus making ASCs stromal or mesenchymal stem cells (MSCs). The International Society for Cellular Therapy (ISCT) has defined minimal criteria in order to define MSCs of human origin: (i) they must be plastic-adherent when maintained in standard culture conditions; (ii) they must express certain markers (such as CD73, CD90 and CD105) while lacking the expression of others (such as hematopoietic markers CD14 and CD45 and human leukocyte antigen HL-DRA); and (iii) they must be able to differentiate to osteoblasts, adipocytes and chondroblasts in vitro (11).

When it comes to the clinical use of adult stem cells, there are some more or less vital criteria that need to be fulfilled. Firstly, it is important that the cells are found in abundance (millions to billions of cells) to provide enough material for cell culture, and it must be possible to harvest them with minimally invasive procedures. The cells should be able to differentiate along several different cell lineage pathways in a reproducible manner and under the control of the scientists. It should also be possible to transplant the cells safely and effectively either to an autologous or an allogeneic host. Lastly, all this should be able to be conducted according to Good Laboratory and Manufacturing Practice (GLP and GMP) (12).

Much of the research conducted with mesenchymal stem cells has been based on MSCs found in bone marrow (BM-MSCs). Especially the differentiation possibilities and immunogenic qualities of these multipotent stem cells make them possible candidates for cell therapy. Unfortunately problems arise, including those concerning harvesting as it can be extremely painful and still tends to result in low cell count. However, it has been shown that ASCs possess similar morphological and immunophenotypical characteristics compared to other MSCs. Isolation success rate, colony frequency and differentiation capacity are similar to other MSCs as well (9,13,14) while they have the major benefit of effortless collection. Based on all these facts, it is clear that ASCs could, at their best, represent a source of stem cells that could enable and result in a multitude of new innovations.

3 General aspects of isolation and differentiation

The ASC isolation techniques used today are originally based on methods developed for rat adipocytes and their precursors in the mid-1960s (15). By altering this protocol first for human adipocyte progenitors (16) and later for ASCs (2,17), we now have a standardized method that is applied routinely. ASCs can be found in and harvested successfully from all adipose tissues around the body (18) but are typically isolated either from subcutaneous samples or liposuction aspirates. In the former case, the sample is minced manually after detachment from the original site, whereas in the latter, the fat tissue is mechanically disrupted at its location within the body by entering the tissue through a small incision with a suction cannula. The fragmentation is further assisted by a collagenase treatment in a 37 °C water bath under shaking conditions. To isolate the stromal vascular fraction (SVF), in which also the ASCs can be found, the digested tissue is washed, centrifuged and filtrated in several steps with appropriate reagents to separate the SVF from the corresponding tissue. Furthermore, ASCs can be derived from SVF based on their plastic adherence property. After isolation is complete, ASCs can be cryo-preserved or plated and cultured as desired.

ASCs can be expanded with relative ease (18) and differentiated toward multiple cell types (1,18) as can be demonstrated by appropriate methods (Table 1), although certain variation in protocols exist among different laboratories. ASCs have the ability to become osteoblasts (i) in the presence of ascorbate, β-glycerophosphate and dexamethasone or vitamin D_3, showing extensive calcium deposits within their ECM. Chondrocyte differentiation (ii), marked by the secretion of cartilage ECM matrix proteins such as proteoglycans, collagens and aggregan, can be induced with transforming growth factor-β, ascorbate and L-proline. With insulin, biotin, panthotenate and dexamethasone ASCs can continue toward what is believed to be their natural pathway and become mature adipocytes (iii) gathering lipids in their vacuoles. Other, even less examined routes are the possibilities of becoming myocytes (iv) of skeletal muscle as well as neuronal-like cells (v), cardiac myocytes (vi) and smooth muscle cells (vii), vascular endothelial cells (viii), hematopoietic support cells (viii), and even pancreatic endocrine (ix) or hepatic cells (x) (18). However, many of these require extensive additional investigation and so far conclusions should be made cautiously.

Table 1. Multilineage capacity of adipose stem cells and methods of differentiation and identification. (19)

Germ layer	Cell lineage	Differentiation stimulants	Histologic and immunohistochemistry lineage confirmation
Mesoderm	Adipogenic	Insulin, 3-isobutyl-1-1-methylxanthine, dexamethasone, indomethacin, ciglitazone	AdipoRed, Oil Red O
	Chondrogenic	Insulin, transforming growth factor β1, ascorbate-2-phosphate	Alcian Blue, collagen type II
	Myogenic	Hydrocortisone	MyoD1-specific antibodies
	Osteogenic	1,25-Dihydroxyvitamin D3 or dexamethasone, ascorbate-2-phosphate, β-glycerophosphate	Alkaline phosphatase, von Kossa stain
	Smooth Muscle	HUVEC culture media, 1% FBS, heparin	Alpha smooth muscle actin, calponin, and myosin heavy chain
Ectoderm	Neurogenic	β-Mercaptoethanol or neurobasal medium supplemented with B27 containing FGF and EGF	Neurogenic proteins (eg, NeuN, GFAP, MAP-2)
Endoderm	Endothelial	Media 199, vascular endothelial growth factor, bFGF, 3% FBS	CD31, CD34, VE-cadherin
	Hepatic	EGF, activin A, bFGF, hepatocyte growth factor, nicotinamide, KnockOut serum replacement	Periodic acid-Schiff base stain

Abbreviations: FBS, fetal bovine serum; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; GFAP, glial fibrillary acidic protein; HUVEC, Human umbilical vein endothelial cells; MAP-2, microtubule-associated protein-2; NeuN, neuronal nuclei; VE, vascular endothelial.

4 Changing the culture conditions – novel approaches

4.1 Use of serum versus use of substitute supplements: going xeno- and serum-free

So far, most of the cell therapy based clinical methods require considerate amounts of cells, which can only be feasible through expansion in vitro when autologous material is used. In the majority of the research using ASCs, FBS is used as the major protein component of the culture medium because of its availability, low price and ability to support cell proliferation, growth and attachment. However, the use of animal-derived components in clinical cell therapies presents significant issues due to safety concerns. There have been reports of severe anaphylactic and other immune reactions (3,4), and the risks of viral and bacterial infections, fungi, zoonoses and prions exist (20). Alternatives have been developed as it would not only eliminate these issues but also remarkably diminish the risk of rejection. Possibly the easiest way to achieve this is by using either allogeneic (21) or, still better, autologous products such as human serum (22), umbilical cord serum (23), or alternatively components such as albumin (24), platelet-rich plasma (25,26) and platelet lysate (27). Then again, these are all human blood-derived products and so are associated with certain limitations: they may not be available for long-term cultures and their composition may vary

considerably between different lots or may not even be definable. To ensure the consistency, efficacy and safety of the procedure, all the steps in the manufacturing process of the final, transplanted ASCs need to be standardized. This leads us to yet another alternative: if we could replace all animal-derived, ill-defined components with purely synthetic XF/SF reagents, we could forget all the aforementioned concerns.

There are only a mere handful of XF/SF supplements on the market today and fully defined cultivation protocols for them are scarce. Moreover, in the XF/SF studies reported, the isolation and even early expansion have often been conducted in serum-containing medium making the effort of avoiding exposure worthless. Taking this into consideration, Patrikoski *et al.* (28) were able to demonstrate that such procedure is in fact feasible and does not appear to affect the end result significantly.

As has been demonstrated (17,28,29), ASCs retain their differentiation ability and phenotypical characteristics under XF/SF conditions with minor differences. Compared to serum-containing medium, they do have considerably shorter doubling times, which would make it possible to speed up the process of propagation. The flow cytometric immunophenotype analysis of ASCs prior to differentiation shows no other significant dissimilarities except the expression of CD54, an intercellular adhesion molecule, and of CD45, leukocyte common antigen. The former can be seen in practice especially when plating the cells: in the absence of serum, the flasks need to be pre-coated. Even then, the very first plating is often critical since not all cells are able to adhere. Cell morphology proves the observation since XF/SF cells appear more spindle-shaped and smaller in size that do the more rounded, almost cuboidal cells grown in medium-containing serum. However, the low expression of CD54 may implicate that the cell population is more homogenous because of the more defined, more selective isolation and expansion protocols. On the other hand, CD45 is a protein tyrosine phosphatase receptor and is expressed on all leukocytes. It regulates the signaling between T- and B-cell receptors and corresponding antigens, and higher concentrations may induce higher immunogenic responses, as discussed below. The XF/SF cultured ASCs may show slightly reduced capability to gather lipids or calcium deposits in staining but, on the other hand, the expression of corresponding cell type-specific genes is higher compared to serum-containing medium (28). Per se, there are signs of differentiation and this may only be due to the nutritional inadequacy of the XF/SF medium. Chondrogenic differentiation, however, appears to be intensified and the size of the grown pellet larger. Although possibly growing with less nutrients, all these differences compared to serum-based cultivation may also be explained by the high proliferation rate. Due to technical reasons, there is plenty of space for the cells to form a large cartilage pellet

but especially the wells under osteogenic induction become rapidly confluent and the already loosely attached cells begin to detach. This happens so quickly that the differentiation only just has time to begin. Regardless, the minimum criteria for defining multipotent mesenchymal stem cells (11) is fulfilled verifying their origin, and so, by improving the current XF/SF culture methods, we might just get the full benefit of its advantages.

4.2 Macromolecular Crowding –method

The term macromolecular crowding refers to the fact that the total concentration of macromolecules inside a cell is so high that the actual volume available for the molecules is significantly less than the anatomical volume of the cell. This is the result of a phenomenon called excluded volume effect: the volume surrounding a certain molecule becomes unavailable for the other molecules and is determined by the molecular mass and shape of the first particle. When the excluded volume is taken into consideration, the effective concentrations of different macromolecules in the solution become larger and thus make their chemical activity increased as well. This in turn affects directly the rates and thermodynamic states of equilibrium of all their reactions. The larger the molecule, the greater the effect will be. In general, the major reactions involved include the binding of macromolecules to surfaces, the formation of macromolecular complexes and aggregates, and the folding of proteins; all in attempt to reduce the total excluded volume and hence the free energy of the system. (30)

Traditionally, the composition of the culture medium has been based on a principle of minimum requirement: using only the reagents vital for the growth and proliferation of the cells while at the same time trying to maintain the process as simple as possible for the cultivator. This however, matches extremely poorly the environment in the natural habitat of the cells. For several years now, there has been a growing interest and a corresponding number of studies exploring different scaffolds and biomaterials to mimic the natural multidimensional surface cells face in living tissues. The flasks and wells used in basic cell culture are flat and smooth, and the effect of surfaces should not be underestimated as mechanical factors are strongly correlated with the success of the culture. Nonetheless, we should inspect the effects of the fluid components at the same time and as eagerly in order to bring the medium up to date as well.

So the minimum requirement of the medium has led us to a situation where the composition is far off the actual, living tissue equivalent. This is especially true with the overall macromolecular concentrations as the serum or plasma derivatives or other protein supplements described above are

alone responsible for the extracellular macro-environment. The high dilution of these components typically results in concentrations around 1-10 grams per liter compared to the much higher values in interstitial fluid (30-70 g/L), blood plasma (80 g/L) or cell cytoplasm (200-350 g/L) (7). The situation is very inconsistent with current data since it has been described on several occasions how the macro-environment affects the formation of ECM through excluded volume and crowding effects, and ECM in turn directly guides the actions of the cells. (7,8,30-32) (Figure 1)

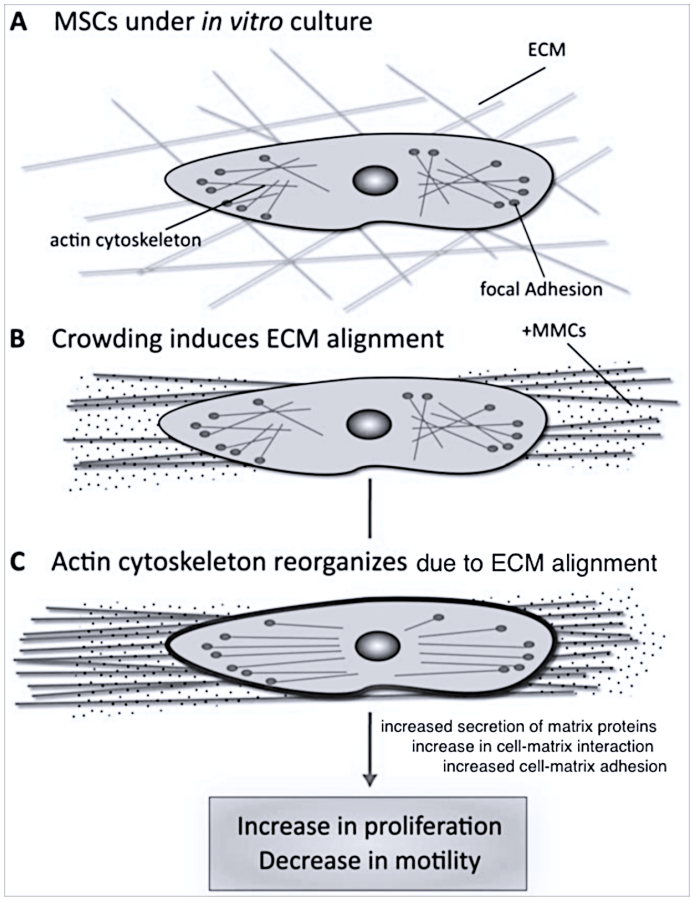

Figure 1. The effect of macromolecular crowding on the reciprocity between the cell and ECM. The crowders promote ECM alignment which in turn affects the organization of the cytoskeleton and protein secretion of the cell. As a result, the overall degree of organization, cell adhesion and cell proliferation all increase. Modified from Zeiger et al (7).

Crowding can be accomplished by several methods. Since serum admixture is the primary responsible effector, it would seem logical to increase its concentration. This, however, has been seen to result in excessive proliferation and other disturbances because of the simultaneous rise in growth factor, hormone and other metabolism-related protein concentrations (7). Neither should it be forgotten that, for clinical purposes, the aim is to replace all animal-derived components, and therefore turn to synthetic materials. It is clear that this way authentic biological conditions cannot be reached, and so the intention is to simply mimic them as well as possible. One such possibly profitable material, and already widely used one, is polyethylene glycol. Unfortunately it has proven to be somewhat problematic since defining the nature of its interactions with proteins has not been possible (30). A polysaccharide called dextran is used as a crowder as well but its problems lie in its electronegative charge. Ficoll (trademark of GE Healthcare companies) then again, is an uncharged, small (diameter of 4-8 nm) and non-cytotoxic polymer of sucrose. It dissolves readily in aqueous solutions without altering their viscosity notably and, because of its neutrality, does not interact or alter the ordinary charge-dependent cellular reactions apart from its crowder function.

Zeiger *et al.* (7) reported the effects of MMC on mesenchymal stem cells by using a mixture of 70 and 400 kDa Ficoll as a crowding agent. With the admixture, the final concentration of the culture medium was set to a level corresponding to that of the protein concentration of blood plasma (approximately 80 mg/mL). They compared intra- and extracellular protein organization between cultures under MMC and without it, and were able to show that crowding itself was able to further the alignment of ECM even in the absence of cellular interaction. The ECM in turn affected cell-matrix interactions and promoted adhesion, thus having an influence on the formation and structure of the cytoskeleton. At the same time, these crowded, more organized cells would begin to secrete more matrix proteins to again enhance its formation in a feedback-like manner. By promoting cell-matrix interaction and adhesion, the crowding also increased MSC proliferation since proliferation has been shown to depend on initial seeding density (33), and at the same time, decreased their ability to migrate (Figure 1).

However informative, the study of Zeiger *et al.* (7) was conducted using animal-derived serum and bone marrow stem cells. Furthermore, it was intentionally purely focused on ECM-cell-interactional mechanisms of undifferentiated cells and did not take a stand on the influence on differentiation. Yet, these are indeed aspects to be considered in cellular therapy. Thus by learning the peculiarities and means of efficient utilization of macromolecular crowding, we could, again, receive more consistent results.

5 Prospects of research

As mentioned, much remains to be examined in regard to clinical potential of ASCs. There are still gaps in our understanding of their essential characteristics, let alone the management of all different aspects together in cell culture. The escalating number of both clinical and pre-clinical studies is extremely encouraging and significant steps have already been taken forward in only a few years.

5.1 An interesting feature under exploration: immunomodulatory properties

As the fundamental goal of ASC-based research is the possibility to treat patients and human diseases, the same components and aspects of treatment should be considered as with any medical remedy. With actual medications, pharmacology is of significance, and it is essential to know how the drug affects the patient and, on the other hand, how the patient affects the drug. By attempting to avoid allogeneic reagents and thus rejection, the question of patient influencing the treatment is

considered, but how about the question of how the treatment – the implanted cells – affects the patient?

One of ASCs' interesting features is their capability to modulate immunogenic responses. Again, this function has been thoroughly proved for mesenchymal stem cells, especially for BMSCs, and ASCs have been much less studied. However, these two do appear to feature mainly the same basic principles as they are weakly immunogenic and able to suppress allogeneic lymphocyte reaction and proliferation (34). Puissant *et al*. (34) summarized the immunological properties of ASCs as follows: (i) they are unable to provoke allogeneic lymphocyte immune response, (ii) they inhibit lymphocyte proliferation, (iii) their inhibitory effect is dependent on the number of ASCs and the duration of contact, both being directly proportional to the inhibition, and (iv) all this requires cell-cell contact to begin but the effect persists even after separation although slightly diminished. The latter observation indicates the fact that the inhibition is not only mediated by cell-cell adhesion but also by secretion of anti-inflammatory substances such as cytokines and chemokines, which may not be spontaneously produced by the ASCs but a result of allogeneic exposure (34,35). Besides these qualities, ASCs lack the expression of major histocompatibility complex class II (MHC-II) molecules and co-stimulatory molecules CD40, CD80 and CD86, the three of them being involved with T- and B-cell stimulation and activity (35), which again makes ASCs regulators of T-cell functions.

In search of more safe and efficient cultivation protocols, it would be beneficial to replace all animal-derived components with more defined ones as described. The immunological properties of XF/SF cultured ASCs were recently explored by Patrikoski *et al*. (35), who discovered that the culture condition does influence their properties in this aspect as well. Cells cultured with FBS were the strongest to suppress immune reactivity but cells cultured with HS or XF/SF supplements did so as well and the differences were minor. However, the result is in line with significantly lower expression of CD54 in XF/SF cells, since this adhesion molecule plays an important role in mesenchymal stem cell-mediated immune suppression. Because of their immunological characteristics, ASCs cultured either in serum-containing medium or XF/SF medium could be worthy candidates for cell therapy. Even if the grafted cells were exposed to animal-derived components during manufacturing, or XF/SF cultured but allogeneic cells were used, immunological disorders and allogeneic conflicts could probably be avoided based on these results. Another possible application could be cell-based immunotherapy: the use of ASCs as a tool to go against inflammatory reactions or even as modulators of existing conventions of the autoimmune system. Like with all applications using ASCs though, it should first be made sure that these cells

are sufficiently controlled. In the case of modulating the immune system, it must be certain that the mechanisms vital and necessary for the patient are not suppressed as well: this might lead to events such as tumor formation or facilitated growth and metastasis even in the absence of uncontrolled differentiation (36,37).

5.2 Clinical achievements and current trials

Being a relatively new discovery, clinical studies aiming to treat patients with ASCs have only begun to be reality. Not that many trials have actually been carried through but the number keeps expanding. The future seems very optimistic since the possibilities of ASCs lie in so many areas of specialty that the number of those interested is tremendous. All the aforementioned characteristics of ASCs combined with the possibility to avoid all the ethical issues faced with embryonic stem cells for example, provide a highly prospective solution to a number of diseases. What is more, it would now seem that the targets of treatment are not limited to tissues of mesodermal origin but include those of ectodermal and endodermal origin as well. An example of all the clinical implications – the currently known ones and those at least hypothesized to exist, that is – was presented by Schaffler *et al.* (Table 2). Different chemical methods, such as macromolecular crowding, as well as mechanical tools, such as vibrations (38) and scaffolds of various materials (39), can be used to modulate and direct the cultivation.

Table 2. An example of some of the possible clinical applications for adipose stem cells. GVHD = graft-versus-host disease. **(18)**

Type of differentiation	Clinical implications
Adipogenic	Breast soft tissue reconstruction after tumor surgery for breast cancer, breast asymmetry, and soft tissue and subdermal defects after trauma, surgery, or burn injury
Chondrogenic	Cartilage repair in joint and disc defects, plastic reconstruction of ear and nose defects
Osteogenic	Skeletal regeneration of inherited and tumor- or trauma-induced bone defects
Myogenic	Tissue reconstruction after trauma and surgery, dystrophic muscle disorders
Cardiomyogenic	Heart muscle regeneration, functional improvement after myocardial infarction, heart failure
Vascular/endothelial	Neovascularization, ischemic diseases
Neurogenic	Brain injury, stroke, peripheral nerve injury
Pancreatic/endocrine	Insulin-secreting cells, type 1 diabetes mellitus
Hepatic	Chronic liver failure, hepatic regeneration, hepatocyte transplantation
Hematopoietic	GVHD, bone marrow support

Trials based on differentiation toward mesenchymal lineages were the first clinical trials to be conducted on ASCs. There have been reports of bone reconstructions (40,41), soft tissue augmentations (42,43) and cartilage applications (44,45), and the potential of ASCs to treat skeletal muscle injuries has also been investigated (46). Some ongoing trials study the possibilities of treating defects such as those of cardiac muscle, vascular tissues, gonads and the urinary tract as well.

Studies describing the use of ASCs in ectodermal conditions are much fewer. These include skin grafts, which have been studied

successfully (47), but there are several trials looking even into the possibilities of repairing neuronal tissue. Currently the means of treatment in neurology are often insufficient and neural tissues themselves are considered to have an extremely poor capability to regenerate, so the newest studies have raised the hopes for many. Endodermal trials, then again, are aiming to find solutions to problems such as those of pulmonary stroma, hepatic and pancreatic tissues, and gastrointestinal tract. (48)

The classical treatment pathway begins form cell implantation and then proceeds to differentiation and finally defect repair. A current trend in cell therapy, however, is the transition from this classical method to the manipulation of existing tissues by means of cellular communication. Through the release of different mediators and transmitter substances the grafted cells could act as conductors and only direct cell behavior in the implantation site. This is where the immunomodulatory effect especially has proven to be very promising. Of all the ongoing clinical trials, Crohn's disease and articular diseases are the most studied ones and there have even been some clinical trials attempting to treat HIV and graft-versus-host disease for example (48). It seems that through immunomodulation inflammation can be diminished and immune system in general altered. As said previously, studies conducted with mesodermal cells are still today most common, but the aspect of cellular communication has notably increased the share of studies conducted with cells of other origins as well.

5.3 Expectations for the future

Therapeutically the possibilities of ASCs lie within several branches of medicine, and as novel aspects of ASCs are still continuously discovered, completely new applications are come up with regularly as well. We cannot yet know which are practicable and valuable in reality as we are still in the middle of the process of understanding the characteristics and requirements of ASCs. If we could understand the basics, we might more efficiently be able to focus on enhancing the procedure with mechanical and chemical techniques. Then again, these two work vice versa, and neither should come to a halt as they reinforce each other. As is natural and fortunate too, further research leads to further knowledge and always awakens new questions. As an end result, we can only conclude that both in vitro and in vivo clinical trials are still required before we are ready to offer ASCs as off-the-shelf treatments in everyday practice. Nevertheless, with all the ongoing research, rapid development should be expected.

6 Conclusions

Until recently, all medical conditions requiring tissue repair or modulation have been treated by either helping the existing tissues to regenerate or by taking grafts from the patient's own corresponding tissues elsewhere in the body. These methods are not always adequate or trouble-free: bone transplants for example are painful, strenuous for the patient and still often insufficient and thus unsuccessful. With ASCs and cell-based therapies, however, it now seems possible to bypass many of these problems all the while being safely and effectively able to repair the damage. Moreover, with ASCs it is possible to create new material instead of having to weigh the advantages of tissue transfer against the many disadvantages. Compared to embryonic stem cells, ASCs do have some limitation such as restriction in differentiation capability, but they also have plenty in their favor. Though limited, their possible courses of differentiation are fairly extensive as they are able to head toward cells and tissues of not only mesodermal origin but ectodermal and endodermal as well. A relatively new yet promising approach is the manipulation of existing tissues through cellular communication, which could provide the solution to many immune disorders and immunologic diseases. Compared to many other cell types previously used in stem cell therapy, ASCs are easy to harvest, isolate and culture in an environment that, for the researcher too, is manageable and modifiable. Finally, their chemical and cyto-architectural characteristics appear profitable. Much remains to be investigated; yet much has already been achieved with the knowledge we have. Off-the-shelf regenerative medical products are not reality today but as we are beginning to understand and slowly master the possibilities of cell therapy in all its forms, we may be in the process of actualizing designs many thought were impossible.

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